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RELATIONSHIP BETWEEN AORTIC AUGMENTATION INDEX AND BLOOD PRESSURE DURING METABOREFLEX ACTIVATION IN HEALTHY YOUNG MEN

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ABSTRACT

Background: Heightened aortic augmentation index (AIx; surrogate of arterial stiffness) is associated with an elevated risk of cardiovascular events; however, it is currently unclear whether peripheral blood pressure modulates AIx. Aim: Given this, we studied the relationship between AIx and blood pressure under resting conditions as well as during skeletal muscle metaboreflex activation, which is a maneuver that generates steadily elevations in blood pressure. Methods: In nine healthy male subjects (23±2 years), the graded activation of the muscle metaboreflex was achieved by post-exercise ischemia (PEI) following moderate and high intensity static handgrip performed at 30% and 40% maximum voluntary contraction. Heart rate (ECG), arterial blood pressure (BP) and AIx (SphygmoCor) were measured. Results: Compared to rest, MAP was significantly increased during PEI30% (+24±4 mmHg, $P<0.05$ vs. Rest) and was further augmented during PEI40% (+34±4 mmHg, $P<0.05$ vs. PEI30%). Similarly, AIx@HR75 increased significantly from rest during PEI30% (Rest -9±3% vs. PEI30% +9±5%, $P<0.05$) and was further augmented during PEI40% (17±4%, $P<0.05$ vs. PEI30%). At rest, there was no relationship between AIx and blood pressure. However, at PEI30%, there was a significant association between AIx and diastolic BP and MAP ($r=0.92$; $r=0.87$, respectively $P<0.05$) and this association was maintained at PEI40% ($r= 0.94$; $r=0.91$, respectively $P<0.05$). Conclusions: Our results indicate that acute elevations in peripheral blood pressure are an important determinant of AIx during muscle metaboreflex activation in healthy men.

Key words: arterial stiffness, metabolic activation, exercise, arterial pressure, aortic blood pressure

INTRODUCTION

Arterial stiffening increases the conduction velocity of both incident and reflected pulse waves, resulting in augmented aortic pressure and ventricular workload [1; 2]. Arterial stiffness increases with age [3; 4], in the presence of chronic degenerative diseases [5-7] and is associated with increased risk of cardiovascular events and death [8-11]. The aortic augmentation index (AIx) has been widely used as noninvasive measure of arterial stiffness [12]. AIx is calculated from central arterial blood pressure applying a generalized transfer function on radial arterial pressure wave form acquired using applanation tonometry [13; 14]. AIx is described as the percentage increase in pulse pressure on the incident wave (first systolic shoulder) caused by the early return of the reflected wave from the periphery [15]. Several chronic disease states are associated with increased AIx [5; 16-19], and chronic structural changes of the vessel wall could be the major contributors [20]. In addition to this, there are other factors such as the distending pressure and vascular smooth muscle tone that may acutely influence AIx [21; 22].

Previous studies have established that vasoactive drugs may acutely change AIx in healthy humans [21; 22]. Systemic administration of nitroglycerin decreases AIx, while it is increased by infusion of either angiotensin II [21] or noradrenaline [22]. These findings may suggest that alterations in peripheral vascular tone play a role in mediating acute changes in AIx. However, on the basis of studies employing intravenous infusion of angiotensin II and noradrenaline, Wilkinson et al. [22] argued that blood pressure *per se* is a more important determinant of arterial stiffness than peripheral vascular tone. In contrast to the significant relationships between AIx and blood pressure observed during infusion of vasoactive drugs [22], previous studies have indicated that resting values of AIx are not associated to resting blood pressure [11; 23]. The reason for this disparate findings might be related to the fact that at low levels of arterial pressure the wall pressure is predominantly support by more compliant elastin fibers, nevertheless at higher levels of blood pressure wall pressure is supported by more stiffen collagen fibers [24; 25]. Nevertheless, the relationship between aortic pulse wave form and peripheral impedance is complex and not fully understood.

An alternative approach to study the relationship between AIx and blood pressure under resting conditions as well as during steadily elevations in blood pressure, would give us further insight on the importance of blood pressure in altering AIx in healthy humans. Muscle metaboreflex evokes robust elevations in blood pressure from rest and can be activated by circulatory arrest of the active limb just before the cessation of exercise and maintaining this during recovery (post-exercise muscle ischemia,

PEI) [26]. With this maneuver, metabolic by-products of muscle contraction are trapped in skeletal muscles and stimulate metabolically sensitive afferents fibers. This results in a steadily elevated blood pressure achieved in part by sympathetically-mediated vasoconstriction, with the heart rate maintained at the baseline levels, evoked by parasympathetic reactivation with the cessation of the exercise [27]. With these in mind, we aimed to test the hypothesis that AIx progressively increases in response to graded metaboreflex activation and that AIx responses are importantly associated to changes in blood pressure.

METHODS

Ethical Approval

All experimental procedures and protocol were approved by Fluminense Federal University ethical committee (CAAE - 09282812.3.0000.5243) and was undertaken in concordance with the declaration of Helsinki. All subjects received a detailed verbal explanation of the intended protocol and a written informed consent was provided and signed by each subject.

Subjects

Nine healthy men with mean age of 23±1 years, height of 179±0.02 cm and weight of 74±2 kg (mean±SE) participated in this study. The subjects were nonsmokers with no history of cardiovascular or other chronic disease and none of them were using any medication. Participants were instructed to abstain from caffeinated, alcoholic beverages, and exercise for at least 24 hours prior to the study, and to have a light meal at least two hours before the protocol.

Experimental Protocol

The subjects were in a supine position on a medical examination table and an electronic handgrip dynamometer held in the dominant hand (model TSD121C; BioPac Systems, Goleta, CA, USA). The maximal voluntary contraction (MVC) was taken as the highest force produced during 3–5 maximal efforts, each separated by at least 1 min. After determining the MVC subjects rested for at least 20 min before the commencement of the protocol. After 3 minutes of baseline, each subject performed 2 min of static handgrip exercise with the right hand at a moderate (30% MVC) and high (40% MVC) intensity followed by 3 min of PEI to isolate muscle metaboreflex activation. The PEI following both 30% and 40% intensity handgrip was used to grade increase the blood pressure. The PEI was achieved by the inflation of a blood pressure cuff around the right arm to suprasystolic pressure of 250 mmHg, 10 s before the end of handgrip exercise, using a rapid inflation unit (E20; Hokanson, Bellevue, WA). To examine the impact of the sympathetic activation during PEI on the radial artery, radial arterial diameter measures

were performed in a subset of subjects (n=5). The diameter was assessed in supine position at rest and during PEI 40% for 20 seconds.

Experimental Measurements

Heart rate (HR) was continuously monitored using a lead II electrocardiogram. Arterial blood pressure was measured in rachial artery on the left arm through auscultatory method by mercury sphygmomanometer, on the second minute of baseline, PEI30% and PEI40%. Peripheral mean arterial pressure (MAP) was calculated as MAP= diastolic blood pressure + (0.4*(Pulse Pressure) [28]. The assessment of arterial wave contour characteristics was performed on left wrist noninvasively using SphygmoCor System (AtCor Medical, Sydney, Australia) in the third minute of baseline, PEI30% and PEI40%, as previously described [14; 29]. Briefly, radial arterial pressure waveforms were record by applanation tonometry on the left wrist. Radial blood pressure waveforms were calibrated from brachial systolic and diastolic blood pressure. A validated, generalized transfer function was used to generate the corresponding aorta pressure waveform [14]. Pulse wave analysis of the aortic pressure wave form provide the following key variables of interest: AIx was calculated as the pressure on the second shoulder (peak pressure) minus the pressure on the first shoulder of the aortic pulse wave divided by the pulse pressure, represented as a percentage. AIx adjusted for heart rate of 75 beats per min (AIx@75bpm), wasted left ventricle pressure energy (Ew), $Ew=[(\pi/4)*(AG \times \Delta tr)*1.333]$. Where: 1.333 is the conversion factor of millimeters of mercury per second to dynes per second per centimeter squared, Δtr is the systolic duration of the reflected wave and AG is the augmented pressure that is the amplitude of the reflected wave and is defined as the difference of the first (forward wave) and second systolic shoulder (reflected wave) of the aortic systolic blood pressure. Only high quality recordings were accepted for analysis (in device quality index > 80%). The assessment of radial arterial diameter was obtained in the nondominant wrist using a Doppler ultrasound (Vivid 7, GE Medical Systems, USA), equipped with a linear array transducer operating at a frequency of 5MHz. Radial artery was imaged at the position that resulted best border visualization and velocity tracing [30; 31]. The Doppler ultrasound video signal was captured at a frequency of 30 Hz using a video capture board with an audio USB 2.0 (Easycap DC60, Leadership) connected to a laptop computer. The video files were compatible with commercial automated edge-detection and wall-tracking software (Vascular Research Tools 5, Medical Imaging Applications), which was used for offline analysis. In the initial phase of software analysis, regions of interest were identified

at the optimal portion of the artery image. An R-wave gating function was not applied to continuously assess radial artery diameter or blood velocity.

Statistical Analysis

All analyses were performed using the Statistical Package for the Social Sciences, version 13 for Windows (SPSS, Chicago, IL, USA). To assess cardiovascular responses to graded muscle metaboreflex activation, Friedman ANOVAs were performed followed by Wilcoxon as a multiple comparison test. The relationship of AIx and blood pressure was evaluated by using a Pearson product moment correlation coefficient. Statistical significance was set at $P<0.05$. Results are presented as means ± SE.

RESULTS

HR was not different from rest during either PEI30% or PEI40% (Fig. 1A). Brachial MAP (Fig. 1B), and brachial and aortic systolic and diastolic blood pressure (Table 1) increased during PEI30% and had an additional increase during PEI40%. Examples of arterial pressure waveforms obtained at rest and during PEI40% condition are shown in Fig. 2.

Compared to rest, AIx was significantly increased during PEI30%, and was further augmented at PEI40% (Fig. 3A). Similar results were obtained when AIx was corrected for heart rate (Fig. 3B). Radial artery diameter did not change during PEI40% when compared to rest (respectively, 2.3±0.1 mm vs. 2.4±0.1 mm; $P>0.05$). The wasted left ventricle pressure energy was higher during PEI, but was similar between PEI30% and PEI40% (Table 1).

Linear regression analysis was used to calculate changes in AIx by a given increment in blood pressure. At rest, there was no relationship between AIx and blood pressure (table 2 and Fig. 4A). However, at PEI30%, there was a significant association between AIx and diastolic blood pressure and MAP ($r=0.92$; $r=0.87$, respectively $P<0.05$; Fig. 4B) and this association was maintained at PEI40% ($r=0.94$; $r=0.91$, $P<0.05$; Fig. 4C). While during rest no association between AIx and systolic blood pressure were found.

DISCUSSION

This present study was designed to investigate the AIx response to graded metaboreflex activation in humans and the modulatory role of the associated increase in blood pressure. The major novel finding of the present study is that AIx progressively increased with graded metaboreflex activation, and that while resting blood pressure was not associated with AIx, during metaboreflex activation a strong association between blood pressure and AIx was observed. Therefore, our findings indicate that acute elevations in peripheral blood pressure are an important determinant of AIx during muscle metaboreflex activation in healthy men.

A plethora of studies indicate that large artery mechanics are complex and mainly determined by its matrix components (i.e., elastin and collagen) and the distending pressure. With the advancing age, disturbance of the relative content of these molecules triggered, for example, by inflammation or hypertension, contribute to increased vessel stiffness [20; 32; 33]. In addition to having structural determinants, arterial stiffness is also affected by vascular smooth muscle cell tone, which is in turn partly regulated by endothelial cell signaling [34]. A contribution from the sympathetic nervous system to arterial stiffness has also been posited but that proposal remains controversial [31]. Previous studies reported a direct relationship between resting muscle sympathetic nerve activity and indexes of arterial stiffness in healthy men [31; 35-37]. Huijben et al [38] indicated that such associations were independent of blood pressure, suggesting that sympathetic nervous system exerts a direct effect on the mechanical properties of the aorta. In contrast, Lydakis et al. [39] demonstrated that despite similar levels of sympathetic activation evoked by lower body negative pressure (LBNP) and isometric fatiguing handgrip exercise, only the latter resulted in an increase in AIx. Since LBNP provokes sympathetic activation without significantly affecting resting blood pressure, the authors concluded that blood pressure rather than sympathetic activation seems to play the major role in modulating AIx. This present study supports and extends these findings with the demonstration that changes in the AIx are highly related to changes in blood pressure during metaboreflex activation (Table 2 and Fig. 4B, C).

Muscle metaboreflex activation was employed in the present study by means of PEI. We found that both blood pressure and AIx were increased from rest during muscle metaboreflex activation at 30% MVC and further increased at PEI40%. Kalfon et al. [18] also reported an increase in AIx in response to muscle metaboreflex activation in overweight/obese men. However, they reported that when muscle metaboreflex was activated concurrently with cold pressor test (additive effect condition) no further

191 increases were observed in AIx, despite greater increases in blood pressure [40]. This is in contrast to our
192 findings and to the concept that blood pressure is an important determinant of AIx during metaboreflex
193 activation in humans. The reason for this disparity is unknown but it could be related to the fact that we
194 have used non-obese men.

195 We reasoned that AIx changes in response to graded muscle metaboreflex activation are
196 importantly dependent on elevations in arterial blood pressure. This contention is based on our finding of
197 the slopes and r-values of the linear regression between AIx and blood pressure. In support of our
198 findings, Wilkinson et al. [22] demonstrated similar linear relationship of AIx with MAP during infusion
199 of noradrenaline and angiotensin II, although the latter had greater effect on peripheral vascular
200 resistance. Of note, lower body negative pressure was associated with a small decrease in AIx, despite a
201 marked increase in vascular constrictor tone and unchanged MAP [39]. One possible explanation is that at
202 low levels of arterial pressure the wall pressure is predominantly support by more compliant elastin
203 fibers, nevertheless at higher levels of blood pressure wall pressure is supported by more stiffen collagen
204 fibers [24; 25]. However, a multitude of complex interacting factors may contribute to aortic pulse wave
205 form and AIx such as, differences on the reflection sites and aortic shape [41]. The aortic pulse wave
206 counter depends on incident wave characteristics as much as reflected wave, while AIx is most
207 determined by arterial stiffness [42].

208 In contrast to the significant relationships between AIx and blood pressure observed during
209 metaboreflex activation, resting values of AIx were not associated to resting blood pressure. In fact, the
210 associations between blood pressure and the AIx at rest are quite controversial in the literature. London et
211 al. [11] found AIx to be related to MAP in patients with end-stage renal failure. In contrast, Wilkinson et
212 al. [23] did not find a correlation between AIx and peripheral MAP in subjects with diabetes mellitus type
213 1. The conflicting results reported in the literature may be attributed to the large variability in the
214 population studied (e.g., clinical condition, age, drug regimens). Furthermore, Nurnberger et al [43]
215 reported a relationship between AIx and diastolic blood pressure, MAP and pulse pressure in healthy
216 young subjects during rest. However in our results AIx was only related to blood pressure during the
217 metaboreflex activation. Therefore, the level of blood pressure could contribute to these results since
218 aortic stress-strain relationship is not linear.

219 Our Doppler measurements indicated that the diameter of the radial artery was unchanged with
220 the PEI, despite the well known increase in sympathetic outflow with the metaboreflex activation our

results are in concordance with previous work that had shown that sympathetic stimulation does not change the diameter of muscular medium-size arteries [44; 45]. Overall, this indicates that our major findings were not affected by diameter changes at the place where the AIx was derived.

Perspectives

Pulse wave reflection is an important factor in determining central pulse pressure. Additionally, central systolic pressure and central pulse pressure contribute to the left ventricle workload and have been shown to be markers of increased risk of cardiovascular morbidity and mortality [46-49]. To our knowledge, this is the first study designed to examine aortic augmentation index in response to graded muscle metaboreflex activation in men. Our current results demonstrate that blood pressure is an important modulator of AIx during metaboreflex activation in men. Together, the present and previous studies provide important physiological insight into arterial-hemodynamic interactions, which appear to have implications for development of cardiovascular risk. In addition, a major factor in the pathogenesis of hypertension is an age-related increase in blood vessel stiffness that results from replacing elastic fibers with fibrous connective tissue. Our results indicate that changes in blood pressure has an influence in AIx and it is likely possible that the changes in blood pressure *per se* play a role in the well-recognized interaction between cardiovascular diseases, aging and arterial stiffness.

Limitations

The limitations of the design and interpretation of the present investigation should be considered. We used a generalized transfer function to indirectly assess aortic wave pressure from the radial artery. Despite this, previous clinical studies demonstrated that this is a reliable tool for precise measure of central arterial blood pressure waveform [13; 14]. Second, we must consider the applicability of the present findings limited only to men, given a previous study [50] indicating a sex-specific relationship between muscle sympathetic nerve activity and AIx. Lastly, although we do not have data on sympathetic nerve activity, it is well known in the literature that muscle sympathetic outflow is steadily increased during metaboreflex activation in men [51; 52]. With this in mind, future studies should investigate whether increases in sympathetic activity are related to increases in AIx during metaboreflex activation in men.

CONCLUSION

In conclusion, our data demonstrate that peripheral blood pressure plays a determinant role in changing AIx during graded metaboreflex activation in healthy men. Thereby, our results indicate that changes in blood pressure should be taken into consideration when interpreting changes in AIx.

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FIGURE LEGENDS

Figure 1. Summary data showing heart rate (panel *A*) and mean blood pressure (panel *B*) at rest and during post-exercise muscle ischemia to 30% (PEI-30%) and 40% (PEI-40%) of maximal voluntary contraction. *Difference from rest (P<0.05). †Difference from PEI-30% (P<0.05).

Figure 2. Examples of pulse waveform at rest (panel *A*) and during post-exercise muscle ischemia to 40% of maximal voluntary contraction (panel *B*) for the radial artery and aorta.

Figure 3. Summary data showing aortic augmentation index (panel *A*) and aortic augmentation index @HR75 (panel *B*) at rest and during post-exercise muscle ischemia to 30% (PEI-30%) and 40% (PEI-40%) of maximal voluntary contraction. *Difference from rest (p<0.05), †Difference from PEI-30% (P<0.05).

Figure 4. The relationship between aortic augmentation index (AIx) and diastolic blood pressure (DBP) at rest (panel A) and during post-exercise muscle ischemia to 30% (panel B) and 40% (panel C) of maximal voluntary contraction.

Table 1. Physiological measurements at rest and during post exercise muscle ischemia.

	Brachial Artery Blood Pressure (mmHg)		Aortic Blood Pressure (mmHg)		
	Systolic	Diastolic	Systolic	Diastolic	Energy Waste (dyn)
Rest	114.1±2.6	67.2±1.6	95.7±2.0	67.7±1.8	-196.0±129.2
PEI 30%	135.9±5.8*	89.0±4.3*	119.4±5.2*	88.5±3.8*	807.9±291.7*
PEI 40%	145.8±3.5*†	100.0±2.8*†	134.1±4.1*†	99.5±3.9*†	1056.4±201.6*

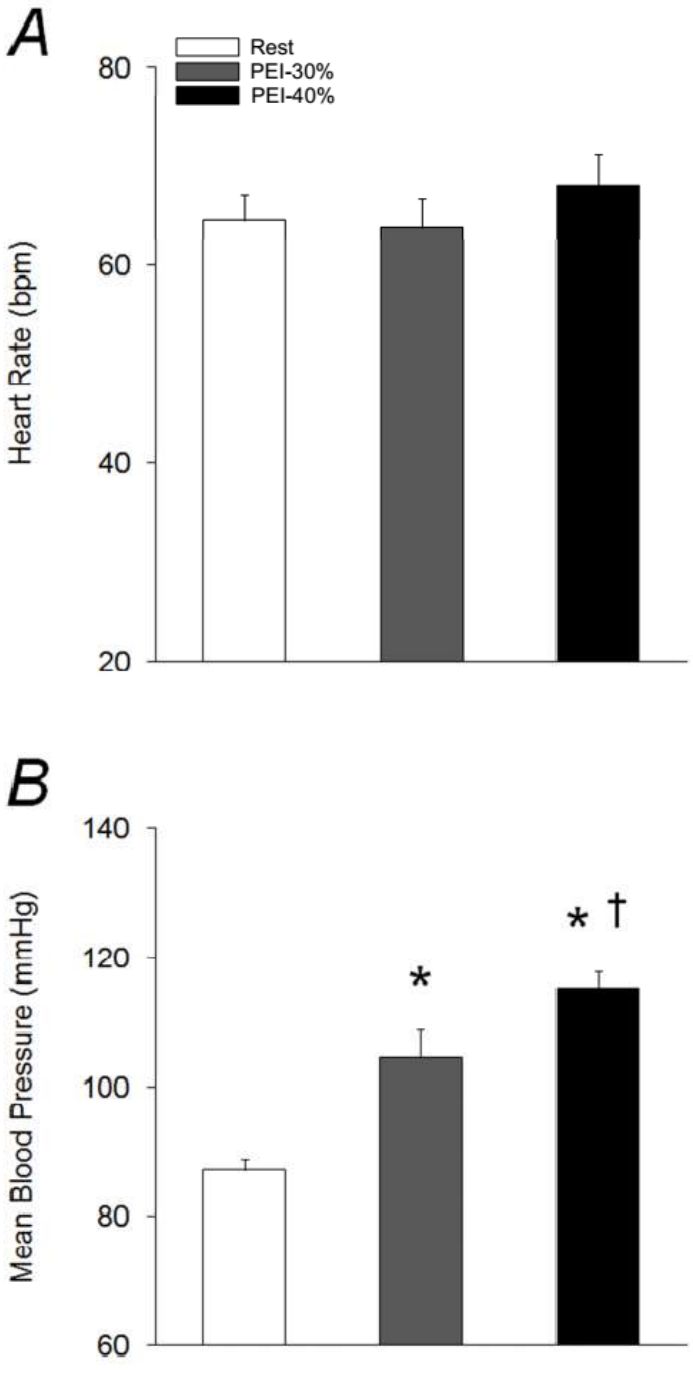
PEI 30%, post exercise muscle ischemia at 30% of maximal voluntary contraction; PEI 40%, post exercise muscle ischemia at 40% of maximal voluntary contraction. *Differences vs. rest ($p<0.05$); †Differences vs. PEI 30% ($p<0.05$).

Table 2. Pearson correlation and linear regression analysis between AIx and arterial blood pressure.

		Rest	PEI 30%	PEI 40%
AIx				
	SBP	y = 0.0662x - 11.693 r = 0.07, P=0.85	y = 0.5252x - 57.267 r = 0.60, P=0.10	y = 0.4883x - 50.711 r = 0.40, P=0.27
	MAP	y = -0.1588x + 9.5021 r = -0.12, P=0.75	y = 0.9583x - 88.764 r = 0.87, P<0.01	y = 1.3003x - 133.05 r = 0.91, P<0.01
	PP	y = 0.2543x - 16.06 r = 0.28, P=0.47	y = -0.168x + 22.455 r = -0.16, P=0.68	y = -0.4472x + 42.399 r = -0.47, P=0.23

PEI 30%, post exercise muscle ischemia at 30% of maximal voluntary contraction; PEI 40%, post exercise muscle ischemia at 40% of maximal voluntary contraction; SBP, systolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure.

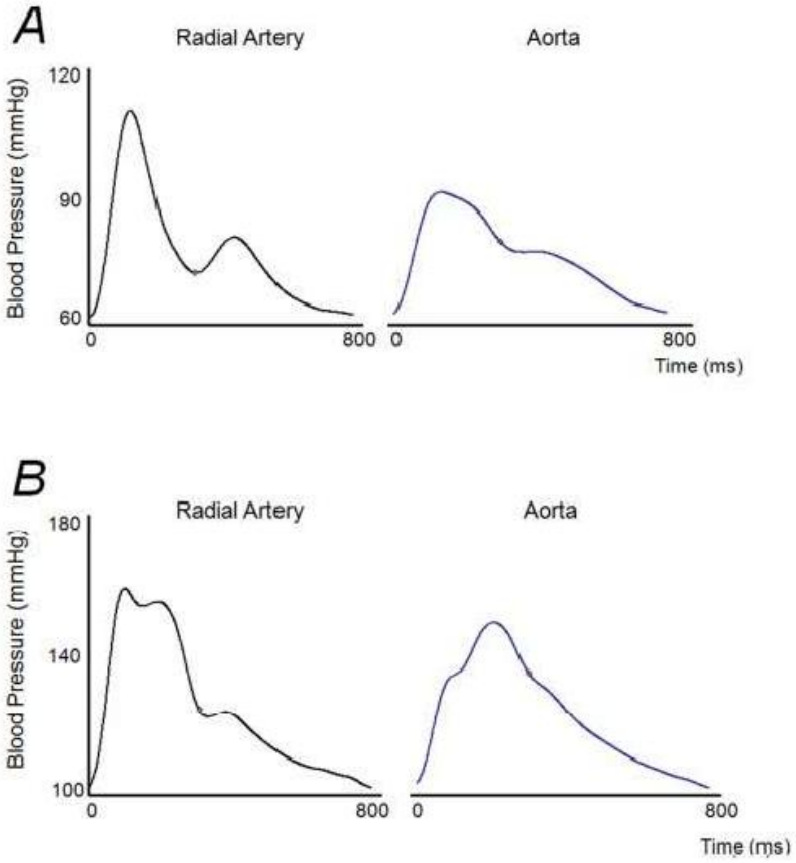
Figure 1.


A
Rest
PEI-30%
PEI-40%
Heart Rate (bpm)
80
60
40
20
B
Mean Blood Pressure (mmHg)
140
120
100
80
60
*
* †

425

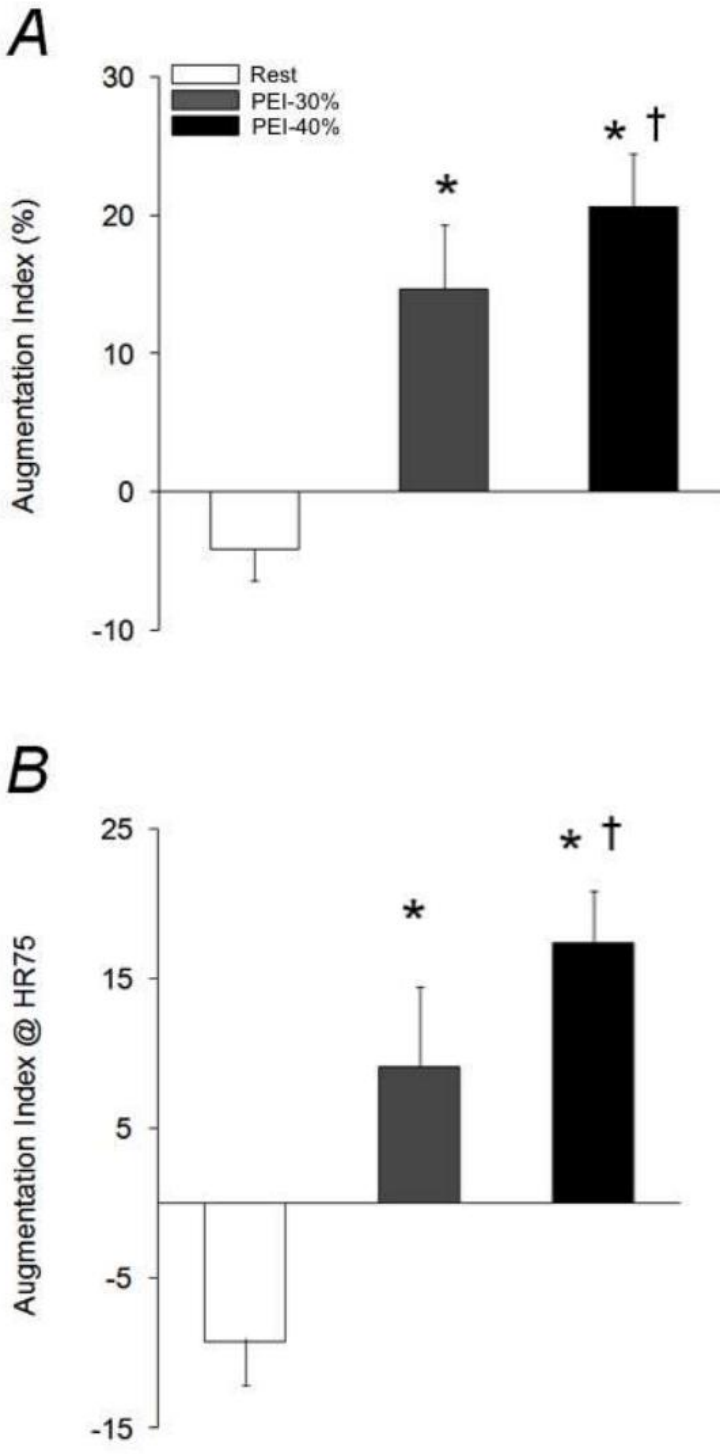
426

Figure 2.



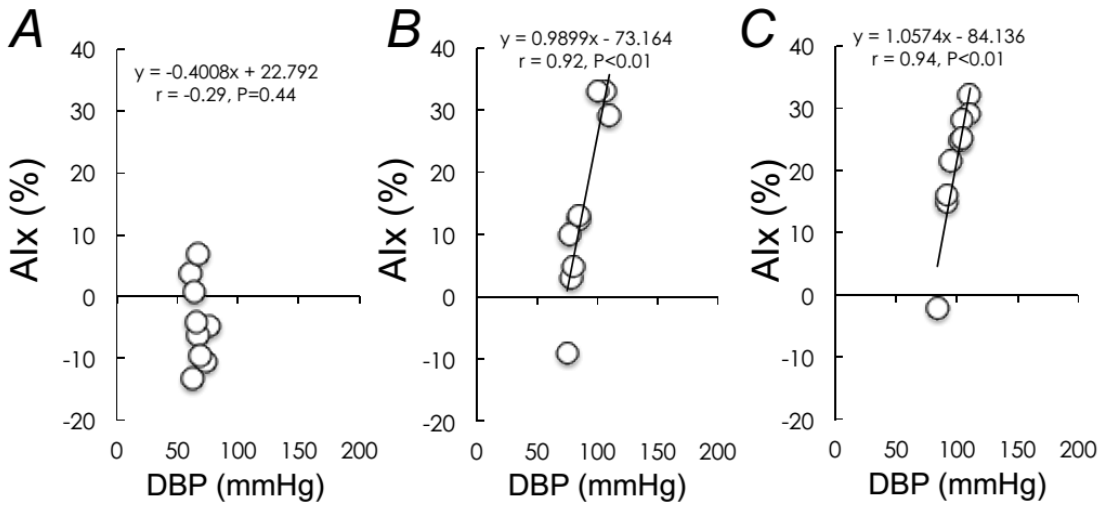
427
428

Figure 3.



429
430

Figure 4


A
y = -0.4008x + 22.792
r = -0.29, P=0.44
AIx (%)
DBP (mmHg)
B
y = 0.9899x - 73.164
r = 0.92, P<0.01
AIx (%)
DBP (mmHg)
C
y = 1.0574x - 84.136
r = 0.94, P<0.01
AIx (%)
DBP (mmHg)